STUDY OF PHOTOSYNTHESIS OF CHLORELLA GROWN UNDER CONSTANT CONDITIONS

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So that photosynthesis of a Chlorella culture as used in systems of biological air regeneration can be controlled, the degree of constancy of photosynthesis must be known when growth takes place under constant conditions [1]. Several investigations have been made of this problem [2, 3], and they have shown that under constant external conditions the fluctuations in rate of photosynthesis of Chlorella vulgaris are small, but over comparatively long periods of time the intensity of photosynthesis undergoes substantial changes. /470

The conditions of cultivation of Chlorella in biological air regeneration systems differ essentially from the conditions of the experiments performed by the authors cited. Because of this, we carried out an investigation to measure the "spontaneous" changes in photosynthesis of Chlorella when cultivated in a special apparatus in which certain of the conditions were maintained at what we consider to be the characteristic level of systems of biological air regeneration (Fig. 1). These conditions were: the presence of a continuous circulation of the suspension of cells in a closed system, the comparatively large volume of Chlorella suspension (7-8 liters), and the possibility of maintaining certain of the cultivation conditions constant.

To stabilize the temperature of the suspension the reactor was fitted with a jacket through which cooling water from the water supply was passed. The flow of water was controlled by the thermometer measuring the temperature of the suspension. With the constant illumination of the culture used in the experiment the error of stabilization did not exceed $\pm 0.3^{\circ}$.

To maintain a constant concentration of carbon dioxide and oxygen in the air bubbled through the suspension, a semiclosed gas system was used (Fig. 2). The flow rate of gas through the gas analyzer was stabilized. The batcher introduced a strictly constant dose of CO_2 into the system in unit time. The design and principle of operation of the batcher will be clear from Fig. 3. It need only be mentioned that the clock controls the valves K_1 and K_2 so that when one valve is open the other is closed.

A distinguishing feature of this system is that, besides measuring the percentage of CO_2 by volume, the gas analyzer also records the intensity of photosynthesis directly, and this greatly facilitates analysis of the experimental results.

If we denote the percentage concentration of CO_2 in the gas discharged from the system by p, the CO_2 supplied from the batcher by q, and the quantity of

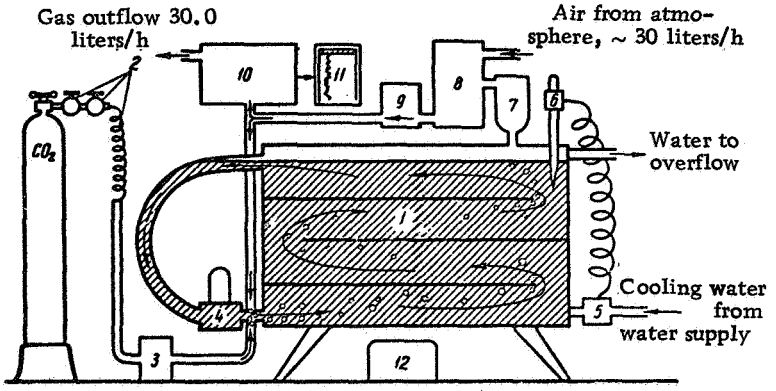

Figure 1. Diagram of Experimental Apparatus: 1) Biological Reactor; 2) Reducing Valves; 3) CO_2 Batcher; 4) Pump; 5) Electromagnetic Valve; 6) Contact Thermometer; 7) Foam Suppressor; 8) Receiver; 9) Membrane Pump; 10) CO_2 Gas Analyzer; 11) Instrument Recording Intensity of Photosynthesis; and 12) Voltage Stabilizer.

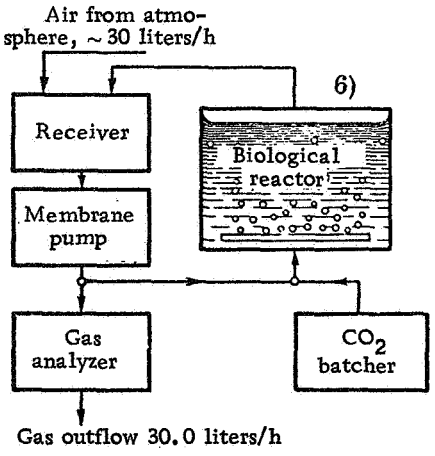

Figure 2. Diagram of Gas System.

gas discharged from the system by Q, we obtain an expression for the intensity of photosynthesis (I_{ps}) determined by the quantity of CO_2 assimilated by the culture in unit time, as follows: /471

$$I_{ps} = q - p\frac{Q}{100}.$$

It will easily be seen that if the values of q and Q are unchanged, the gas analyzer can measure the intensity of photosynthesis directly on a certain scale.

A list of the conditions of growth of the Chlorella as they were stabilized in the course of work with the apparatus, the level at which they were maintained, and the calculated values of the stabilization errors of these conditions and the changes produced by them in the intensity of photosynthesis are given in Table 1.

TABLE 1. Level of Photosynthesis of Chlorella under Different Conditions of Cultivation

Stabilized conditions of growth of Chlorella	Level of maintenance of conditions	Error of maintenance of growth conditions, %		Change in intensity of photosynthesis due to error of maintenance of growth conditions, %	
		Regular	Random	Regular	Random
Intensity of illumination of culture (type 3 N-8 incandescent lamp), lux	20000		±10		±1,33
Temperature of Chlorella suspension, °C	39,5		±0,3		±6,8*
CO_2 concentration in bubbled air, %	2,5 (By volume)	±5	±3	±1,25**	±0,75
O_2 concentration in bubbled air, %	22,3 (By volume)		±0,45		Small
Intensity of aeration, liters/m	10		±3		±3
Intensity of mixing of suspension (output of pump), liters/min	45		±2,5		Small
Mineral feeding	Tamiyas	—1			"
Density of culture of Chlorella cells, cells/cm³	$150 \cdot 10^6$	+30	±1	—7,5**	±0,25
Percentage of photosynthetically active cells in culture***	80		±6,25		±1,2
Concentration of Hydrogen Ions in the nutrient medium (pH value)	8,5		±6		Small
Error of apparatus measuring intensity of photosynthesis					±1

*This value also allows for the error of measurement of the conditions of solution of CO_2 in the mineral nutrient medium during changes in temperature and the associated additional change in photosynthesis.

**Regular errors in measurement of photosynthesis due to change in density of culture and to change in CO_2 concentration is bubbled air were allowed for during analysis of the experimental results.

***Cells measuring 3 and 4 μ and half the number of cells measuring 2 and 5 μ.

Commas represent decimal points.

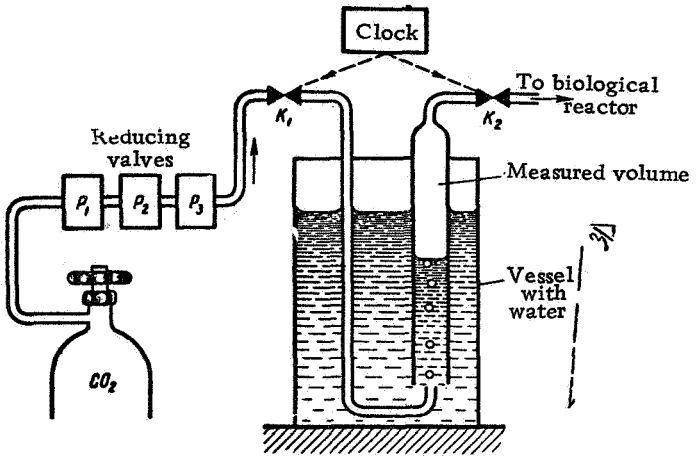

Figure 3. Diagram Showing Construction of Batcher.

The overall standard random error of measurement of photosynthesis in these experiments, calculated as the statistical mean of the random errors listed in column 7 of Table 1, was 7.75%. /472

During analysis of the experimental results, changes in the intensity of photosynthesis by a value less than this error were disregarded, but changes exceeding the error were considered as "spontaneous" changes in photosynthesis.

The curves of photosynthesis recorded by the apparatus were not smooth but contained many random fluctuations (Fig. 4). It was assumed that these fluctuations are due not to changes in photosynthesis, but to random deviations of the growth parameters from their stable values. This assumption was tested and confirmed by a series of experiments carried out on nutrient medium without seeding the culture (Fig. 5). On this basis, for the analysis of changes in photosynthesis under investigation, the curves recorded by the apparatus were first smoothed out.

The mean duration of the individual experiments was 8-10 h. Three experiments lasted 50 h each. Differences in the character of the "spontaneous" changes in photosynthesis in the longer experiments were not significant compared with the shorter. The total duration of the experiments was 320 h. Analysis of the data in the course of the experiments showed that a further increase in the number of experiments does not introduce significant changes into the mean values obtained. Accordingly, a comparatively short total duration of the experiments was all that was necessary.

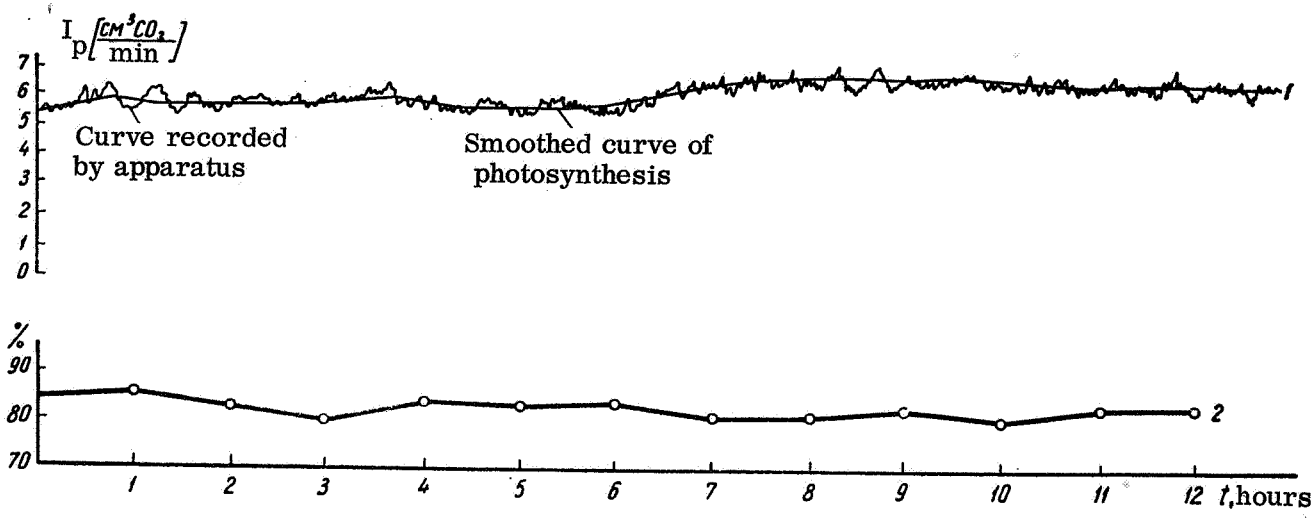

Figure 4. Example of Recording of Changes in Intensity of Photosynthesis (1) and Percentage of Photosynthetically Active Cells in a Chlorella Suspension (2) in Time.

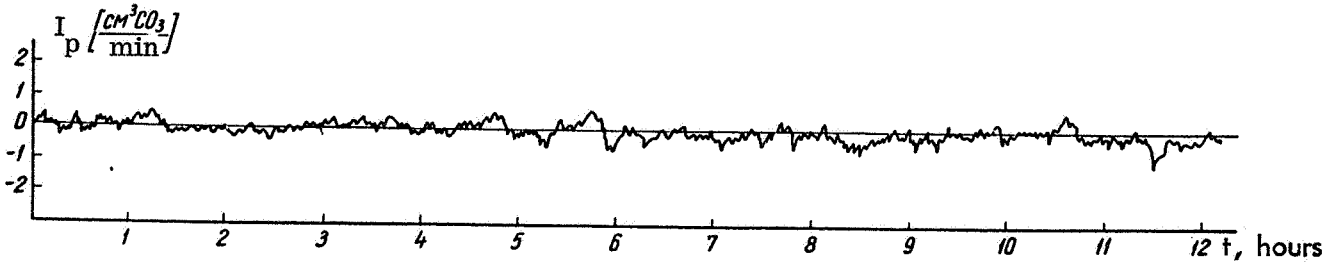

Figure 5. Recording of Intensity of Photosynthesis of Tamiya's Medium Without Seeding of Culture.

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